Preparation of New Benzothiazoles. Pharmacological Action of Benzothiazole and of Some Derivatives.

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DEDICATION

This work is affectionately dedicated to my dear parents in appreciation of their constant encouragement.

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Preparation of New Benzothiazoles. Pharmacological Action of Benzothiazole and of Some Derivatives.

Introduction

Through the Ferguson Fellowship and similar means, the Department of Organic Chemistry of Columbia University sponsors and encourages research which correlates Chemistry with other sciences. Every two years, a Ferguson Fellow is appointed to carry on investigations and research in synthetic drugs and medicines. It was in this capacity that the author began the study of some compounds containing sulphur in the divalent state, namely: benzothiazole, and derivatives of this mother substance, among which were dehydrothio-p-toluidine, 2-phenylbenzothiazole, 2-anilinobenzothiazole, 2-aminobenzothiazole, and 2-phenylbenzothiazole 5-carboxylic acid,—the thiazole isomer of 2-phenyl cinchoninic acid (Atophan).

It was hoped that the data obtained from a study of compounds having such widely differing substituents in the benzothiazole nucleus might bring out some chemotherapeutic relationships or rules for this group.

ABSTRACT

These studies have led to the following original contributions:

A. Science of Chemistry. The synthesis of
  1. 2-phenyl-5-cyanobenzothiazole,
  2. 2-phenylbenzothiazole-5-carbamide,
  3. 2-phenylbenzothiazole-5-carboxylic acid,
  4. the Methyl ester of 2-phenylbenzothiazole-5-carboxylic acid,
  5. Nitro-2-phenyl-5-cyanobenzothiazole.

  The following known compounds were prepared by new methods:

  6. o-aminothiophenol hydrochloride, and
  7. 2-anilinobenzothiazole.

B. Science of Pharmacology. Some reactions of the benzothiazole nucleus
  1. In general: parenterally and subcutaneously,
  2. On respiration,
  3. On circulation,
  4. On muscle,—heart, conjunctivum, gastrocnemius,
  5. On blood uric acid.

A few correlations have appeared between the chemical constitution of the compounds studied and their pharmacological action, namely:

  1. Substituents in positions 2:6 vs. vesicant action,
  2. A substituent in position 2 vs. antiseptic action.

HISTORICAL SECTION

A detailed history of the outstanding contributions of Synthetic Organic Chemistry to Medicine has been clearly presented by Evers.[34] The evolution of our present ideas on the subject of Chemotherapy has paralleled the isolation of active principles from tissues of plants or animals, the elucidation of the chemical constitution and formulae of those principles, the synthesis of the same or of analogous compounds or derivatives thereof, and the biological study of many of these products as well as of some apparently unrelated substances, such as colloidal metals.

The attempts of scientists to correlate chemical constitution with biological action have been only partly successful. Dyson[33, 38] has given a fairly recent summary of such correlations and their bearing on the synthesis and use of new drugs. Since large doses of drugs are usually toxic, although small doses may be very beneficial, it has been necessary to establish a standard of efficacy and safety through the therapeutic index. Thus, if the ratio of the maximum tolerated dose to the minimum curative dose be five or more, a drug may be considered to be of practical value. As certain groups or groupings do not always carry the same drug effects, it is not possible to predict the pharmacological action of new drugs of known chemical constitution.

With reference to known compounds, however, four generalizations can be made. Physiological activity is usually found[33, 34, 36, 38] in compounds having (a) unsaturated linkages, (b) alcoholic or phenolic groups (possibly amino groups), (c) substituents in the 1:3:4 positions in the benzene nucleus, and (d) high molecular weight with fairly good solubility. Examples are (a) neurine, (b) ethanol and phenol, (c) adrenaline, and (d) Bayer 205.

To a very limited extent, eight other correlations can be made, depending on (1) stereoisomerism, (2) spatial arrangements, (3) increasing molecular weights or length of side chains, (4) specificity of groups, (5) detoxifying action of carboxyl groups, (6) the ease of reduction of compounds in vivo, (7) the compensatory activation by hydroxy groups para to carboxyls, and (8) the location of unsaturated bonds or of substituents.

Besides these chemical limitations to the progress of Chemotherapy, there may be others of a physical nature such as cyclic alterations of reactivity as described by Norris:[37] or still others of a biological nature such as specificity of action independent of known conditions, as indicated by Wells[36] on the Chemistry of Immunity. The technical limitations of testing biological activity may also be mentioned here, including not only inadequate methods, but inability of some organisms to utilize or decompose certain compounds.

The subject of Chemotherapy is still in the developmental stage, therefore, and dependent upon the accumulation of additional chemical and pharmacological data for its systematization.

In fact, research in this direction is very important and would constitute a definite contribution to science. The study of anhydrobases as possible synthetic drugs or medicines, or of some of their derivatives for the same has been reported in the literature. Thus, Bogert and Corbitt[26] discovered toxicity and trypanocidal action in the complex 6-amino-2-phenylbenzothiazole-p-arsonic acid. Hunter[31] has referred to the urticarial condition which developed in some of his co-workers while handling some of the benzothiazoles and their precursors. Blum[13] has shown that 2-aminobenzothiazole has a slight antiseptic action.

Additional syntheses of benzoselenazoles and of benzothiazoles structurally analogous to known drugs, such as cinchophen[24] and camphor derivatives,[20, 28] have been carried out. A few correlations have been made here, such as the effect of acetylating p-amino groups,[26] differing the esters of cinchoninic acids, and modifying their 2-phenyl group.[20]

Although chemists have pointed out the structural relationships between these synthetic compounds and drugs already in use, very little pharmacological work has been done to establish the presence of physiological action in them. This has been due in part to insolubility or instability of the compounds. The ideal compound for such work is, of course, one which is fairly easily soluble in water, and nearly neutral in reaction.

Attention is drawn to the interest of another investigator in benzothiazole, namely: Dr. S. W. Clausen, of the University of Rochester, who asked for a sample of our benzothiazole to use in a clinical study of eczema.

The history of the discovery of benzothiazole itself by A. W. Hofmann and its subsequent preparation by other investigators has been ably reviewed by Bogert and Meyer[18] and by Bogert and Stull.[28] Of the various methods at our disposal for its synthesis, aside from those of purely theoretical interest, that of Bogert and Stull was the most promising from the standpoint of yield. Any of the procedures necessitates purification of the base by distillation either directly or following precipitation as a salt. Thus, Möhlau and Krohn[11] distilled the base from an alkaline solution after precipitating it as the double salt with potassium ferrocyanide in acid solution. Mills[19] distilled it from alkaline solution after separating it as the nitrate, or distilled it in the presence of copper. Bogert and Stull[28] carried out the distillation at first in a vacuum, and finally at atmospheric pressure. The yield obtained by Möhlau and Krohn was only 10%, and the method involved the generation of malodorous and poisonous gases which were not readily removed by the available hood. It was believed, on the other hand, that the method of Bogert and Stull would give equally good yields and be much pleasanter to work with in quantity. Such indeed proved to be the case.

DISCUSSION

In attacking this problem, numerous obstacles were encountered, chief among which was the extreme sensitivity of the author to benzothiazole. Not only was the oily base violently irritating soon after coming into contact with the skin, but its accumulation in the system prolonged the effect for some weeks after contact with the compound had been discontinued. In this connection, it is of interest that the derivative, 2-aminobenzothiazole, in saline solution caused only a very slight rubefaction and itchyness of the skin of the author. The transitory nature of this effect as compared with the lasting effect of benzothiazole may be attributed to the solubility of the amino compound in water.

No irritation was experienced in the preparation of the cyanobenzothiazoles. The preparation of the benzothiazole isomer of cinchophen, 2-phenylbenzothiazole-5-carboxylic acid, has been completed, as planned, and its pharmacological action will be studied in the near future. The use of a new compound for the additional investigation of drug action means that more than enough for a representative sample must be prepared, which requires considerable time.

While 2-phenyl-5-cyanobenzothiazole can be prepared by methods directly analogous to those used for the synthesis of 2-phenylbenzothiazole, it is notable that the former does not have the Rosenkörper odor, nor any other.

In part of the pharmacological work, the author had to work alone, which is a severe handicap in doing animal experimentation. Furthermore, the compounds which had to be studied presented difficulties with reference to their solubilities. Thus, benzothiazole is insoluble in water or dilute solutions of salt; the nitrate is soluble in water only in concentrations which produce high acidity and the base separates on dilution or partial neutralization; and the 2-amino compound, while soluble in physiological saline solution, does not give a solution of high concentration. One advantage is obtained with this compound, however, in that the pH value is almost the same as that for blood.

With regard to the outlook for further research in the benzothiazole field, probably the most promising part is found in the allergic phenomena. The anaphylactic symptoms, specific sensitization following an incubation or latent period, and the violence of some of the reactions would warrant the study of the action of epinephrine, or ephedrine, and atropine in counteracting the vasomotor disturbances. Another line of research would be the study of sensitivity in individuals of known pathological condition, such as tuberculosis, pneumonia, hay fever, asthma, arthritis, and so forth. In a recent study,[41] for instance, it was found that allergic conditions were associated with arthritis in 18% of the cases studied, while only 4% of the apparently healthy individuals were sensitive. In similar ways, it might be possible to discover some relation between disease and benzothiazole idiosyncrasy.

The observation of sensitivity in man to dehydrothio-p-toluidine may have some industrial importance since workers on benzothiazole dyes have been known to suffer from skin irritations. This may also explain the otherwise unaccountable occurrence of eczema or urticaria in persons who prove to be wearers of materials dyed with dehydrothio-p-toluidine colors.

The similarity of the benzothiazole dermatitis and latent period to that of poison ivy is remarkable. A higher degree of specificity is apparent in the former, however, as seen when correlated with complexion.

The point of racial tolerance to the action of a drug systemically or locally (on the skin) has already been established for cantharides. Thus, the rabbit is almost immune to the same. This would probably explain why the cutaneous test with benzothiazole as made on rabbits in the laboratories of the Chemical Warfare Service was practically negative, while ours on man were so definitely positive.

The observations on the action of the benzothiazoles on the central nervous system and on the kidneys of animals are of experimental interest although of no apparent practical value. Additional studies are planned to determine the nature of the action on the kidneys, as to whether the urine was suppressed, or

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the vesicle reflex inhibited, and whether this in turn was due to a general circulatory effect, to a local capillary action, or to a direct action on the secreting cells. The base, benzothiazole, may prove to be of some value in the production of experimental nephritis.

The experiments on derivatives of benzothiazole have been too limited to lead to any correlation between chemical structure and physiological action. A few remarks, however, may be made. The replacement of the 2-H in the benzothiazole by 2-NH_2 has not destroyed the antiseptic action, but has rendered the irritant action negligible. The action on respiration, circulation, and muscle, on the other hand, have remained almost unchanged.

Replacement of the 2-H by aniline or phenyl residues appeared to diminish the toxicity of the nucleus for mice, according to our results from subcutaneous injection. Insufficient data have been reported in the literature to furnish an explanation or analogy for this loss of activity in benzothiazole derivatives and other heterocyclic compounds. Dyson states that phenylthiourea is toxic as is thiourea itself. A study of the lipoid/water solubility coefficients of these compounds might shed light on the matter.

It is important that the introduction of a 6-methyl group into the benzothiazole nucleus and a p-aminophenyl group into the 2-position has yielded a compound which still has definite action (i.e., the irritant action of dehydrothio-p-toluidine). The trypanocidal action of the more complex derivative, 6-amino-2-phenylbenzothiazole-p-arsonic acid, is significant when compared with other cyclic arsonic acids.

The reputed similarity of the quinoline nucleus to benzothiazole through the replacement of the 1:2-ethylene linkage by divalent sulphur has been partly substantiated by our results on the study of blood uric acid changes following the administration of a benzothiazole. Possibly the dependence of the activity of cinchophen on its 2-phenyl substituent may be paralleled in the benzothiazole series, barring any biological variants. Our work with 2-phenylbenzothiazole 5-carboxylic acid in the future may thus be very fruitful.

Browning, Cohen, Ellingworth, and Gulbransen[42] have just published some comparative studies of quinolines and benzothiazoles with reference to their trypanocidal and antiseptic action. Chemotherapeutic studies on benzothiazoles corresponding to those of Sasaki and Ueda[43] on quinolines might bring out even more correlations.

EXPERIMENTAL PART

A. Chemical Section

The synthetic chemistry was directed toward the preparation of 1. benzothiazole itself, and 2. of 2-phenylbenzothiazole-5-carboxylic acid.

In view of the fact that 2-aminobenzothiazole is soluble in water and stable in Ringer's solution it appeared to be the best representative of the benzothiazole series for pharmacological investigation. Unlike benzothiazole, this compound is on the market and procurable in pure state at a reasonable price from R. F. Hunter, of London. (M.p., 129.8°C, cor., in agreement with the literature. Anal: Cal. for $C_7H_6N_2S$, C 56.00%, H 4.00%. Found C 56.30%, H 4.33%.)

o-Nitrophenyldisulphide.

The Bogert modification of the Wohlfahrt method was used. Yield: 60%. M.p., 195°C, cor. This agreed with the reports of Bogert and Stull[28] and of Wohlfahrt.[14]

Zinc salt of o-aminothiophenol.

The method of Bogert and Snell[23] gave an 88% yield: reported yield, 90%. This compound turned dark grey at 242°C and shrank and continued to sinter up to 349°, when the charred material melted to a brown tar.

2-Phenylbenzothiazole.

This compound was prepared by the method of Bogert and Stull.[28] Yield: 53.8%: reported yield 61.5%. M.p., 114.2°C, cor. Reported m.p., 114° C[8, 28]: 115°C.[3]

o-Aminophenyldisulphide.

The method of Bogert and Snell[23] gave the reported[29] yield of 57%: and m.p. of 93°C, cor.

2-Hydroxybenzothiazole.

One hundredth mole of the zinc salt of o-aminothiophenol was condensed with two hundredths mole of chlorcarbonic ester by refluxing for two hours. The fused mass was extracted with normal sodium hydroxide solution and then with 50% alcohol. This solution, when neutralized, yielded white needles. On purification, these melted at 136.5-138°C, cor. They were soluble in concentrated ammonia, being again precipitated on acidification. The yield was 10%. The solubilities agree with those in the literature,[2] the reported melting point being 136°C.

2-Anilinobenzothiazole. New Method.

An adaptation of the method of condensing amines with isothiocyanate was used here. One hundredth of a mole of o-aminophenyldisulphide was refluxed with 0.02 mole of phenylisothiocyanate, either with 95% alcohol as a solvent or without it, on the water bath for 40 minutes. Hydrogen sulphide was evolved, and a small amount of precipitate formed in the alcoholic solution. Evaporation of the alcohol and purification from toluene gave a 30% yield. It formed fine white needles. M.p., 159.2°C, cor. In the literature, it is reported as 159.0°C.

Anal. Calc. for $C_{13}H_{10}N_2S$: C 69.03%, H 4.43%.
Found: C 68.88%, H 4.71%.

Hydrochloride of o-aminothiophenol. New Method.

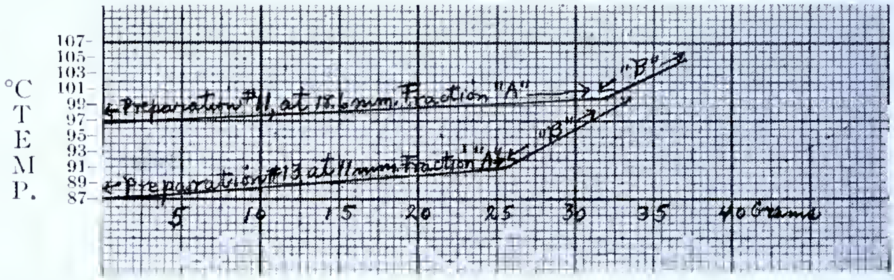
When 8.5 grams of the zinc salt of o-aminothiophenol were treated with 75 cc. of hydrochloric acid (sp.gr., 1.20), there was a slight odor of hydrogen sulphide, and some heat given off due to the solution of the zinc salt. It was filtered through asbestos while still warm, and then cooled in ice. White crystalline needles separated at once. These were filtered off and recrystallized from concentrated hydrochloric acid, filtered, and washed with ether. They were then crystallized from water. M.p., 217°C., cor., decomposing as reported by Bogert and Stull[29] and by Hofmann.[9] The yield of these fine white needles was 80.3%.

Benzothiazole.

According to Möhlau and Krohn,[11] the yield of benzothiazole from fusing sulphur with mono- or di-methyl aniline followed by repeated distillations was only 10%. It was thought that a larger yield might be obtained from o-aminothiophenol hydrochloride and formaldehyde, according to the method of Bogert and Stull.[29] Claasz claimed to have obtained benzothiazoline when he used this procedure originally, but the former authors were unable to corroborate this.

The chief difficulty with this method, as with all others, lies in the distillation which is necessary for purification. Owing to the high boiling point, distillation of the first product at atmospheric pressure is accompanied by considerable decomposition. At diminished pressure, frothing and superheating are prevented only by the use of a Ladenberg flask, capillary tube for admitting fine bubbles of air for stirring the viscous liquid, and small pieces of porous tile.

The purity of the preparations used for pharmacological investigation is shown graphically below.



In preparation No. 11, 32 grams of the pale yellow, viscous liquid were obtained (fraction A) between 97-100°C, the pressure being 18.6 mm.; and 5 grams were obtained at 101-5° (fraction B).

In preparation No. 13, 25.5 grams were obtained between 87 and 91°C, (fraction A), at 11 mm.; and 8 grams between 91-100° (fraction B).

In physical properties, our preparations (fractions A) agreed with those reported by Hofmann,[3] Möhlau and Krohn,[11] and Bogert and Stull,[29] that is benzothiazole is a pale yellow, oily liquid, heavier than water, highly refractive, having an odor like quinoline, and a burning taste; also soluble in ether, alcohol, and strong mineral acids. The specific gravity at 22°C, was approximately 1.23. $N_{D}^{24.6°C}$ 1.6370.

The boiling point at atmospheric pressure (760 mm.) was determined on "Fraction A" in each case, and was 231°C, cor., by the Siwoloboff method.[7] This agreed with that reported by Hofmann[4] and by Bogert and Stull.[29] These fractions were used for the pharmacological tests. Some of 11A was also sent to the Chemical Warfare Service, while some of 13A was sent to Dr. Clausen in Rochester.

The yield of benzothiazole from the thiophenol was 44.2%: from the o-nitrochlorbenzene, 19.8%.

Benzothiazole-methyl iodide.

Fraction B of preparation 11 was used for the synthesis of the methyl iodide derivative by the method of Hofmann.[9] M.p., 210°C, cor., with decomposition, as reported by him.

Microanal. Calc. for C_8H_8NSI: N 5.06%. Found N 5.08%.

Benzothiazole nitrate.

Fraction B of preparation 13 was used for the preparation of the nitrate by Mills' method.[19] The white crystals were obtained as reported, but since the aqueous solution was highly acidic, it was unsuited for pharmacological work, and hence, not prepared in quantity.

In the synthesis leading to the preparation of 2-phenylbenzothiazole-5-carboxylic acid, the starting material was p-chlorobenzonitrile (Eastman).

4-Cl-3-NO$_2$-1-CN benzene.

By the Mattaar method,[22] p-chlorobenzonitrile was nitrated at room temperature with nitric acid (sp. gr., 1.60) in nine hours. Odorless, lustrous white spangles were obtained, from alcohol or toluene. Yield: 60%. M.p., 98-99.5°C, cor. Mattaar gives 99-100°C.

Microanal. Calc. for $C_7H_3O_2N_2Cl$: C 46.03%, H 1.64%, N 15.34%.
Found: C 46.60%, H 1.63%, N 15.17%.

2-NO_2-4-CN-phenyldisulphide.

An adaptation of the method of Wohlfahrt as modified by Bogert and Stull[29] was used here. The canary-yellow disulphide formed at once, so refluxing was unnecessary. The finely granular product was washed with boiling water and then with boiling alcohol. Yield: 86%. M.p. about 335°C, with gradual charring from 235°.

Microanal. Calc. for $C_{14}H_6O_4N_4S_2$: C 46.65%, H 1.67%, N 15.64%.
Found: C 46.92%, H 1.68%, N 15.56%.

Zinc salt of 2-amino-4-cyanothiophenol. $(NCC_6H_3NH_2S\text{-})_2\,Zn$.

The method of Bogert and Snell[23] slightly modified was used. Owing to the insolubility of the cyanodisulphide in boiling glacial acetic acid, the reduction was carried out by refluxing a mixture of the powder and zinc dust (previously ground in a mortar) with the acid for an hour. It was noted that unless extremely pure zinc dust were used (Kahlbaum's), the yield was very small. The powdery product was slightly cream colored. Yield: 49%. M.p. above 363°C, shrinking and charring starting at 250°.

Microanal. Calc. for $C_{14}H_{10}N_4S_2Zn$: C 46.28%, H 2.75%.
Found: C 46.48%, H 3.39%.

2-Phenyl-5-cyanobenzothiazole. $NCC_6H_3NSC.C_6H_5$.

This compound was prepared by the method of Bogert and Stull[28] by treating 2.55 grams of the zinc salt of 2-amino-4-cyanothiophenol in 100 cc. of boiling glacial acetic acid with 2 cc. of benzaldehyde, and refluxing for 30 minutes. The zinc salt dissolved quickly, then the solution turned dark red. Removal of the zinc by hydrogen sulphide and dilution gave a yellow precipitate which was purified from 95% alcohol with Norite. Thin white platelets were formed having a metallic luster. Yield: 30%, the chief loss occurring in the recrystallization. M.p., 196.6-197.7°C, cor.

Microanal. Calc. for $C_{14}H_8N_2S$: C 71.18%, H 3.39%, N 11.86%, S 13.59%.
Found: C 71.16%, H 3.52%, N 11.76%, S 13.42%.

By the method of Bogert and Snell[23] fusion of 7.5 grams of the zinc salt with 12 cc. of benzoyl chloride on the water bath for 30 minutes gave a 20% yield of the same compound. M.p., 197.0°C, cor.

In comparison with 2-phenylbenzothiazole itself, it is notable that this new compound has no odor (Rosenkörper or otherwise).

2-Phenylbenzothiazole-5-carbamide. $H_2NOCC_6H_3NSC.C_6H_5$

The nitrile group of the above compound was partly hydrolysed by heating 3.0 grams of the same in 80 cc. of 75% sulphuric acid at 80°C for ¾ hour. After cooling, the brown solution was poured into 900 cc. of distilled water and the bulky white precipitate was purified by repeated crystallizations from 95% alcohol with Norite. Small white needles were obtained. Yield: 23%. The compound was insoluble in N NaOH solution. M.p., 247.2°C, cor.

Microanal. Calc. for $C_{14}H_{10}ON_2S$: N 11.02%, S 12.59%.
Found: N 10.91%, S 12.15%.

Nitro-2-phenyl-5-cyanobenzothiazole.

Nitration of 2-phenyl-5-cyanobenzothiazole was carried out by treating 0.5 gram with 20 cc. of nitric acid (sp.gr. 1.6) at 25°C for 18 hours. Solution took place at once. The product was precipitated by pouring the solution on ice. For purification of the curdy, pale yellow substance, a large volume of boiling 95% alcohol was needed, and crystallization initiated by the addition of distilled water to the hot solution just to incipient turbidity. A fine white powder was obtained. Yield: 10%. M.p., 256°C, cor.

Microanal. Calc. for $C_{14}H_7O_2N_3S$: C 59.78%, H 2.49%.
Found: C 59.66%, H 2.71%.

Attempted bromination of 2-phenyl-5-cyanobenzothiazole.

In his study of the bromination of benzothiazoles and of naphthathiazoles having substituents in the 2 or 3 position, Hunter[32] found that in general they formed somewhat stable di- or tetra-bromides, or, by loss of varying amounts of bromine, formed more stable 6-bromo derivatives. On the other hand, he found that bromination of benzothiazoles having substituents in the phenylene nucleus resulted in compounds which were usually very unstable, rapidly losing all of the bromine. Results of the latter type were obtained in our research when we attempted to brominate 2-phenyl-5-cyanobenzothiazole. Similar results were obtained by Bogert and Abrahamson[21] in attempting to brominate 2-phenylbenzothiazole. Thus the treatment of 0.75 gram of our compound in 20 cc. of chloroform with 3.2 grams of bromine in 10 cc. of chloroform at room temperature yielded at first a yellow precipitate which then redissolved and formed another precipitate which was vermilion colored. After refluxing for 5 minutes, and rapidly filtering by suction, this product was dried on a porous tile in a vacuum desiccator over potassium hydroxide. This and the small amount of residue left on the filter paper in the air rapidly lost the deep color and in 18 hours became pale yellow. Longer exposure caused no apparent further change in either case. The crude material gave a positive qualitative test for halogen (Beilstein), but on crystallization from absolute ethyl alcohol it lost its bromine, no longer giving the test.

A second experiment was made in which a larger proportion of solvent was used and a smaller amount of bromine, the solution being refluxed for one hour. Evaporation of the solvent and crystallization of the residue from 95% alcohol gave crystals which melted at 194°C, almost the same as the original compound, and showed only traces of halogen. In neither case was there evidence of the rearrangement of bromine to form a stable hydrobromide.

2-Phenylbenzothiazole-5-carboxylic acid. $HOOCC_6H_3NSC.C_6H_5$.

This was prepared from the corresponding carbamide and from the cyano antecedent. Thus 0.29 gram of 2-phenylbenzothiazole-5-carbamide was dissolved in 10 cc. of hydrochloric acid (sp.gr., 1.19) by heating. In a few minutes, the reaction was complete, the new acid forming in a mass of white, felt-like crystals. These were filtered, washed with water, and dissolved in 10 cc. of N NaOH solution, followed by precipitation by N/10 sulphuric acid. At maximum precipitation, the pH was 2.0. The gelatinous acid was repeatedly dissolved in alkali and reprecipitated by acid, and finally crystallized from 95% alcohol, with Norite. There was a contamination with ash which could be removed only by crystallization from absolute alcohol. Yield: 70%. M.p., 273°C, cor., with previous sintering.

Microanal. Calc. for $C_{13}H_8NS.COOH$: (-COOH) 17.6%. Found 18.0%.

Hydrolysis of 2-phenyl-5-cyanobenzothiazole, 0.51 gram, with 10 cc. of sulphuric acid (containing 3 parts by weight of acid to one of water) was carried out by heating the solution to 80°C for 15 minutes, then to 205°C for 15 minutes. Dilution with ice water, after a test sample gave no precipitate with sodium hydroxide solution, and crystallization of the curdy white precipitate from 95% alcohol (Norite), gave an odorless white powder. (See reference No. 21.) Yield: 80%. M.p., 273°C, cor., decomposition beginning at 250°.

Microanal. Calc. for $C_{14}H_9O_2NS$: C 65.88%, H 3.53%, N 5.49%.
Found: C 65.76%, H 3.60%, N 5.55%.

Methyl ester of 2-phenylbenzothiazole-5-carboxylic acid.

A solution of 2-phenylbenzothiazole-5-carboxylic acid was made in dilute sodium hydroxide solution and then treated with an aqueous solution of silver acetate. After acidification (litmus) with nitric acid, the silver salt was filtered, washed with water and alcohol, and dried. It was then refluxed in a methyl alcohol solution of methyl iodide for ten minutes, filtered, and then cooled and partly evaporated. Finely granular white crystals were obtained. Yield: 5%. M.p., 171-2°C, cor., with previous sintering.

Microanal. Calc. for $C_{15}H_{11}O_2NS$: N 5.20%. Found N 5.38%.

EXPERIMENTAL PART

B. Pharmacological Section

Pharmacological experiments were made with

1. Benzothiazole and some of its derivatives,
2. Benzothiazole nitrate, and
3. 2-Aminobenzothiazole.

In view of the fact that benzothiazole is the mother substance in this series, it was studied first. As has already been mentioned, when the author was preparing the base, she had been a victim of its severe vesicant action. She had also observed that two persons who had volunteered to handle the base—one a brunette young woman, the other a blonde young man—were immune to its action. Accordingly, a sample of the benzothiazole was sent to the Chemical Warfare Service for further testing, while our own experiments were being conducted to determine whether there were any correlation between the action and sex, or complexion.

The test was made by placing 0.02 cc. of the oily fluid (preparation No. 11A), representing approximately 25 milligrams, on the flexor surface of the left wrist of the subject. This was allowed to be absorbed by the skin,—which required about fifteen minutes—after which time the characteristic odor was no longer perceptible. A positive reaction consisted of a dermatitis venenata, the redness, swelling, and pruritis involving an area of at least two centimeters square. Forty-three people were tested thusly,—five of whom were females; and twenty-two blondes. Five of the total positive reactions appeared in 36 hours, the remaining twelve being delayed for 9 or 10 days. The latter, however, were equally definite.

Our results disclosed a high degree of susceptibility in relation to complexion. Thus, 46% of the blondes tested gave positive reactions, while only 33% of the brunettes did so.

Although the actual number of females tested was much less than that of the males, the percentage of positive reactions was nearly the same for each. Thus, 40% of the females and 39%

of the males reacted positively. Correlating susceptibility with sex, therefore, showed no marked tendency for females over males.

The similarity in the appearance and behavior of the eruption and the duration of the latent period of the benzothiazole dermatitis to that of poison ivy is noteworthy. A difference, however, may be seen in the results of Krause and Weidman[30] who made clinical tests with poison ivy and found no variation in susceptibility due to complexion.

Besides the remarkable phenomenon of delayed reactions, another surprising feature came to light; namely, the increase of hypersensitiveness following repeated application of the poison. This was seen not only in the author but in a subject who had no further contact with the base. Thus, in him, the application of benzothiazole to the right wrist after the reaction on the left one had partly subsided caused in addition to the direct action, a highly intensified redevelopment of the dermatitis at the original spot on the left wrist.

The cumulative action of the base and its slow excretion prolonged its effects in the author for several weeks after contact with it had ceased. Wet dressings of boric acid solution proved to be the most efficient antidote, though slow. The excessive nervousness which accompanied the poisoning disappeared completely with the other symptoms.

Similar studies made with dehydrothio-p-toluidine gave evidence of this irritant and delayed action in 18% of the number of cases tested (45), although in a milder degree. Here again the blondes were more susceptible than the brunettes.

Neither 2-phenyl- nor 2-anilinobenzothiazole showed such properties.

This specificity of the irritant action for man was emphasized through the tests made by the Chemical Warfare Service of the U. S. War Department. In making cutaneous tests with benzothiazole on rabbits, they found the base inert. This species of animal has long been known to be practically immune to another irritant,—cantharides, while man is highly sensitive to it.

The insolubility of benzothiazole in water and in physiological saline solution (0.9% NaCl) together with its oily consistency rendered it difficult to investigate by the usual pharmacological methods. An emulsion of the base in autogenous serum proved to be the most suitable vehicle for intravenous administration. The results are summarized below.

In its general action after intraperitoneal injection, in the cat, rat, and rabbit, the pure base caused salivation, lachrymation, asphyxial movements accompanied by vasodilation, paralysis, and coma. In the rat, to these symptoms were added anuria, increased irritability, and nystagmus. Whether the anuria were due to an action on the kidney itself, or to a suppression of the urination reflex, remains to be determined. In view of the fact that in our studies on the frog (below), the motor reflexes were depressed, it seems probable that renal depression may account for the anuria.

Following intravenous injection, the respiratory center in the cat was depressed, and in every fatal case, exitus lethalis was due to paralysis of this vital center. The blood pressure was always lowered, but the pulse remained strong even after respiration had been markedly depressed. Autopsies showed vasodilation only. In the frog, no demonstrable action on the heart was noted. In the turtle, the action was apparently directly on the heart muscle, but the depression was so slight that it might be disregarded. In view of this absence of any action on the heart, it seems probable that the circulatory changes were due either to a depression of the vasoconstrictor center or to some undetermined peripheral action.

Some minimum lethal doses are as follows:

	Cat			Rat	
M.L.D.		100 mgs.x k.	M.L.D.		200–300 mgs.x k.
	I.V.			I.V.	
	Rat			Rat	
M.L.D.		1 G.x k.	M.L.D.		3 G.x k.
	I.P.			Os	

Preliminary tests by the method of Reddish[39] gave evidence of antiseptic action toward Staphylococcus pyogenes aureus, the phenol coefficient being 1.77. The occurrence of such action in 2-aminobenzothiazole has already been reported.[13]

Subcutaneous injection of olive oil solutions of 2-anilino and of 2-phenylbenzothiazole into mice (1.2 mgs. x k.) caused no reaction.

The pharmacological investigation of benzothiazole nitrate was not carried on to any great extent, owing to the high acidity of its aqueous solution (0.6N), and also to the fact that the base separated on partial neutralization. In the cat, the respiratory center was immediately depressed. The pulse remained strong after an initial fall and recovery. It was found, however, that the fall in blood pressure after the administration of the drug was very little more than that caused by control tests with acid of the same strength ($0.6N HNO_3$). In the turtle, there was no appreciable action on heart muscle. The general action in one cat after intramuscular injection was primarily an overstimulation of the respiration. Salivation, emesis, and micturition were the paths of excretion of the compound. Paralysis was followed by death in two hours.

Hunter has described 2-aminobenzothiazole[30] as being soluble in dilute acid and in water. Chemically, this compound is closely related to benzothiazole itself, and presumably, like other amino compounds, its pharmacological action would not differ markedly from that of the mother substance. It is soluble in 0.1 N hydrochloric acid solution to the extent of 2%; and in physiological saline solution to 0.2%. The former solution has a pH value of 2.3; the latter, 7.8. Some pharmacological experiments were made with each of these solutions.

Attention has already been drawn to the fact that the rubefacient action of this compound is practically nil.

In the study of the general action of the agent, the administration of 50 mgs. per kilogram of cat, intraperitoneally, produced mydriasis, ataxia, convulsive movements (asphyxial?), winking, licking of the chops, lack of response to pressure, and rapid and shallow breathing. Later on, there were spasms of the muscles of the extremities, and increased reflex excitability. Some animals had "running convulsions."

A dose of 70 mgs. per kilogram of animal produced similar symptoms together with salivation, opisthotonus (asphyxial?), spasm of the extensor muscles of the arms, back and neck, followed by more marked and continuous depression. There was exaggerated reflex excitability. The animals recovered within twenty-four hours.

In the rabbit, whether the compound was in acid or saline solution, its administration promptly produced collapse, paralysis of the hind extremities, and stupor. During recovery, mydriasis came on gradually, as did ataxia, nystagmus, and spasms of the jaws and head. Vasodilation was noted in the ears. The respiration became extremely rapid and shallow. Running convulsions appeared after the administration of the acid solution. Where the reaction was severe, the corneal reflex was depressed. Animals receiving 50 mgs.x k., intraperitoneally, recovered in three hours. The toxic action, therefore, was one of depression and narcosis.

As in the benzothiazole experiments, respiratory and blood pressure changes were studied on anesthetized animals through the trachea and the carotid artery, respectively, and the injections were made into the left femoral vein very slowly by gravity, syringe, or Murphy drip, all records being made on the kymograph in the usual way. One exception was made in experiment No. 9, below.

Of the eight cats used, six were anesthetized with sodium-secondary-butylbromallyl barbiturate (Pernocton*), 80 mgs. x k., I.P.; and two with diethyl barbituric acid (Barbital), 360-500 mgs. x k., per os. Each of the four rabbits used was anesthetized with isoamylethyl barbituric acid (Amytal), 80 mgs. x k., I.P., in N/2 sodium hydroxide solution.

The diluted hydrochloric acid solution of the agent was administered to four of the cats; the saline solution to the other four and to the rabbits.

The range of therapeutic dosage was also studied at this time. Considerable difficulty was encountered in administering the saline controls and the agent itself, owing to the frequent formation of clots in the cannulae, both arterial and venous. The effect of 2-aminobenzothiazole on clotting-time remains to be determined.

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* Acknowledgment is here made of the very generous provision of Pernocton for this work by the firm of Riedel-de-Haen, Inc., New York.

Since a dilute acid was to be used as a solvent, it was necessary to determine the effect of the acid on the rate and depth of breathing.

The respiratory changes as recorded graphically have been analyzed below. At selected intervals, the rate of breathing has been counted, and the extent of the excursion (depth of breathing) measured. Some of these results are given in tabular form.
Thus, it was found that neither small nor large doses of N/22 HCl had any apparent effect on the rate; however, doses larger than one cubic milliliter per kilogram produced a very great increase in depth (at least 100%) and, of course, in ventilation also (at least 200%).

In experiment No. 2, it will be noted that aminobenzothiazole-hydrochloric acid caused an acceleration of breathing from 5 per minute to as much as 23 (360%), while the depth of breathing was regularly decreased. It is worthy of note that the effect on ventilation was comparatively slight. In the light of experiment No. 1, it is evident that aminobenzothiazole stimulated the rate of breathing at the same time that it produced a very marked decrease in its amplitude. Large doses killed by respiratory paralysis.

In a third experiment, a male cat weighing 4.25 kilograms was anesthetized with barbital, and given the drug in saline solution intravenously. Thus, the complication of an acid solvent was absent. Nevertheless, the effect of aminobenzothiazole on the respiration was in the same direction as in experiment No. 2.

In this series, (Experiments 1, 2, and 3), the depth was altered only slightly, while the rate of breathing was increased much more and consequently the total ventilation showed a primary stimulation, followed, after large doses, by a secondary depression.

When given slowly, the maximum therapeutic dosage was between 16 and 18 mgs. x k. of animal.

In experiment No. 9, a male rabbit weighing 3 kilograms was anesthetized with (50 mgs. x k.) amytal, I.P. The respiration was

Acid Control

Experiment #1. *Male Cat: 3.23 K.*

Pernocton: 80 mgs. x k., i.p.

TIME AFTER ANESTHETIC		INJECTIONS SUBSTANCE			RESPIRATION		VENTILATION
Hrs.	Mins.		cc.xk.	cc.x min.	Rate	Ampl. mm.	
3	34				8	12	96
	35				12	12	144
	36	N/22 HCl	0.6	4	12	—	
	37	do.	0.6	4	10	—	
	38	N/10 NaHCO$_3$	0.6	1.3	11	—	
	39	N/22 HCl	0.9	7.5	11	—	
	40				12	3	36
	41	(Pointer readjusted)			12	25	300
	42	N/22 HCl	1.5	3	13	25	325
	43	N/10 NaHCO$_3$	1.5	5	—	25	
	48	N/22 HCl	4.0	4	11	52	572
	49				10	52	520
	50				12	50	600

2-Aminobenzothiazole dissolved in N/22 HCl.

Experiment #2. *Female Cat: 3.34 K.*

Pernocton: 80 mgs. x k., I.P.

TIME AFTER ANESTHETIC		INJECTIONS SUBSTANCE			RESPIRATION		VENTILATION
Hrs.	Mins.	(A = Agent) mgs. x k.	cc.x k.	cc.x min.	Rate	Ampl. mm.	
3	32				4	25	100
	33				5	20	100
	34				5	20	100
	35	A 7	0.6	7.5	8	17	136
	36	A 10.5	0.9	6	12	12	144
	37				23	4.5	103.5
	38				21	5	105
	39				18	12	216
	41	N/10 NaHCO$_3$	0.6	3	17	13	221
	42				13	12	156
	43				14	12	168
	44	A 10.5	0.9	5	14	12	168
	45	A 17.5	1.5	6	13	9	117
	46	N/10 NaHCO$_3$	1.5	7.5	11	5	55
		Total 45.5	Death from respiratory failure.				

studied by means of a pleural cannula, and the agent was administered intraperitoneally, the solvent being saline.

Sixty milligrams per kilogram of animal produced an initial stimulation of the rate of breathing, then a slowing, followed by a gradual, partial recovery. The total change, however, was small. The amplitude was altered to a greater extent in being increased, decreased, and then increased again. The ventilation was similarly affected. Death was due to respiratory paralysis after the total dosage of 160 mgs. x k.

It may be mentioned here, that with large doses, unanesthetized animals developed the same symptoms of respiratory depression as did those under anesthesia, even when the agent was administered intraperitoneally.

2-Aminobenzothiazole dissolved in 0.9% saline.

Experiment #4.

Male Rabbit: 2.0 K.
Amytal: 80 mgs. x K., I.P.

TIME AFTER ANESTHETIC		AGENT	INJECTIONS SALINE	(FEMORAL V.)	RESPIRATION		VENTILATION
Hrs.	Mins.	mgs.x k.	(Solvent) cc.x k.	cc.x min.	Rate	Ampl. mm.	
4	28				18	27	486
	29				16	28	448
	30		0.5	1.5	16	28	448
	31				15	29	435
	32	1	0.5	1.5	15	30	450
	33				20	31	620
	34		0.5	3.8	16	38	608
	35	1.7	0.9	5.0	17	42	718
	38		1.5	6.0	13	38	494
	39				18	37	665
	40	3	1.5	3.8	24	30	720
	41				22	32	704
	42		2.5	4.5	22	33	726
	43				20	32	640
	44				21	33	693
	46	5	2.5	5.4	25	28	700
	47				29	21	609
	48				30	18	540
	49				31	17	557
	50		5.0	3.0	28	20	560

2-Aminobenzothiazole dissolved in 0.9% saline. (Continued)

TIME AFTER ANESTHETIC		AGENT	INJECTIONS SALINE	(FEMORAL V.)	RESPIRATION		VENTILATION
Hrs.	Mins.	mgs.x k.	(Solvent) cc.x k.	cc.x min.	Rate	Ampl. mm.	
	51				27	20	540
	55				27	22	594
	58	10	5.0	1.3	—	28	
5	—				25	22	550
	6				25	17	425
	10				39	11	429
	11		10.0	2.3	29	14	406
	44				23	17	391
	47	17	8.5	1.6	23	23	529
	48				30	18	540
	49				28	14	392
	53				26	11	286
	56				20	12	240
	59				15	23	345
6	—				12	24	288
	1				2	37	74
	2				0	0	
	3				0	0	
	4				0	0	
	5				0	0	
	6				0	0	
		Total 37.7					

Small doses (3-17 mgs.) seemed to increase the rate and depth of breathing, while larger amounts caused slower and shallower breathing.

Thus, in both the cat and the rabbit, 2-aminobenzothiazole in small doses caused an acceleration of breathing, and in large doses a marked decrease. This is in accordance with the well-known rule for other drugs, that there is usually a reversal of the effect with increasing dosage. With regard to the depth of breathing, small doses caused a slight increase, while large doses caused a marked decrease.

In almost every case, with very large doses, death resulted from respiratory failure. These results have been confirmed in unanesthetized rabbits.

The effect of 2-aminobenzothiazole on circulation will now be considered. Tracings of blood pressure and pulse rate were made simultaneously with those of respiration. These have also been analyzed and tabulated numerically.

2-Aminobenzothiazole dissolved in 0.9% saline.

Experiment #5. *Female Cat: 2.65K.*

Barbital: 260 mgs. x K. per Os
100 mgs. x K. periton.

TIME AFTER ANESTHETIC		INJECTIONS (FEMORAL V.) AGENT	SALINE		BLOOD PRESSURE LEVEL	PULSE RATE
Hrs.	Mins.	mgs. x k.	cc. x k.	cc. x min.	mm.	per 10 sec.
4	37				140	45
	38		0.05	1.7	138	42
	39	0.1	0.05	1.0	132	40
	40				132	42
	41				132	39
	42		0.5	0.9	132	42
	43	1	0.5	1.0	132	39
	44				130	44
	51				82	28
	52		0.5	1.4	90	43
	53	1	0.5	1.0	88	42
	54				86	41
	57				92	42
	58		1.5	1.5	94	45
	59				98	46
5					100	45
	10	3	1.5	2.5	118	37
	11				122	32
	15		2.5	0.8	126	36
	21				104	30
	22	5	2.5	2.1	106	32
	23				106	30
	24				106	33
	25				110	29
	26				110	27
	34		5.0	1.1	120	37
	42				138	39
	43				140	38
	48	10	5.0	2.0	124	35

2-Aminobenzothiazole dissolved in 0.9% saline. (Continued)

TIME AFTER ANESTHETIC		INJECTIONS (FEMORAL V.)			BLOOD PRESSURE LEVEL	PULSE RATE
		AGENT	SALINE			
		mgs. x k.	cc. x k.	cc. x min.		
Hrs.	Mins.				mm.	per 10 sec.
	49				139	37
	52				126	36
	53				102	38
6	16		5.0	1.6	84	27
	21				116	31
	22	10	5.0	1.4	114	30
	28				116	26
	37				84	30
	45		10.0	3.0	101	32
	51				86	26
	54	20	10.0	1.4	101	27
7	11				116	30
	31		15.0	1.6	104	29
	52	30	15.0	1.4	118	29
8	10				96	30*

* This animal was chloroformed next day.

The pulse was slowed by aminobenzothiazole only very gradually, and to the extent of 33% only after very large doses. The blood pressure was lowered temporarily but this was followed by rapid recovery. After very large doses, it was lowered about 28%.

In experiment No. 9 (see page 31), sixty mgs. x k., of rabbit caused a marked fall of blood pressure and a primary (compensatory?) acceleration, followed by a secondary slowing of the heart. It would appear that the fall in blood pressure were the essential action on the circulation, the decrease in the pulse rate being due to inefficient coronary circulation. Large doses paralyzed the vasomotor mechanism, the heart beating for some time after the blood pressure had fallen to zero.

The effect of 2-aminobenzothiazole on heart muscle was studied in the turtle. The saline solution of the agent applied directly to the surface or injected into the auricular or ventricular cavity, had practically no effect on the rate or strength of beat, in the normal heart or in that artificially depressed by chloral hydrate. In the latter case, the agent produced a negligible rise in rate.

In the study of the general action of 2-aminobenzothiazole, it had been noted in the cat and rabbit that the muscles were now soft, now hard; in all probability this was due, not to a direct action on the muscle cells, but to an action on the central nervous system. The spasm was most marked in the cat when the animal gave exaggerated responses to noises (calling, jarring table and so forth).

These effects were studied further and analyzed on striated muscle in the frog. The experiments were of two types. In the first, a tetanizing current was used to stimulate the gastrocnemius muscles submerged either in a bath of "normal" Ringer's solution alone or in the same containing the agent (0.2% strength).

In the control, the rate of onset of fatigue in the 5-minute interval between the third and fourth stimulations (10 to 15 minutes) was (4 mm., or) 0.8 mm. per minute. With the agent, in one case (experiment No. A), it was 3.4 mm. per minute; and in another (experiment No. B), 4.25 mm. per minute. In other words, the muscle in the agent was fatigued from 4 to 5 times faster than that in pure Ringer's solution. Furthermore, the muscle thus poisoned did not recover readily.

In the second type of experiment, an induction current was used to stimulate the muscle under definite conditions in each of the above-described baths. The results are tabulated below.

In the control, the total decrease in response after seventeen stimuli, or 47 minutes, was 13 mm., i.e., 0.27 mm. x min. The agent caused a total decrease of 40 mm. in the same time interval and with the same number of stimuli; i.e., 0.85 mm. x min., which is approximately three times greater. During the first five minutes of contact of the muscle with the agent, there was a depression of 2.2 mm. x min., in contrast to 0.4 mm. x min. for the control.

This initial depression was thus about 5.5 times greater than normally occurred. At the end of 18 minutes, it was nearly ten times greater. Placing the poisoned muscle in pure Ringer's solution again, and allowing a 5-minute rest period was inadequate

0.2% solution of 2-Aminobenzothiazole in "Normal" Ringer's solution.

Experiment #6.

Frog: gastrocnemius muscle.
Induction current (6cm.).
10 grams afterload.
Duration of stimulus; 5 sec.

R = pure Ringer's sol.

R & A = Agent in Ringer's solution.

STIM.	TIME INTER-VAL	CONTROL BATH	RESPONSE		TEST BATH	RESPONSE		AVERAGE DEPRESSION—MAKE CONTROL	TEST
#	min.		Make mm.	Break mm.		Make mm.	Break mm.	mm. x min.	
1	0	R	73	72	R	40	61		
2	2	R	75	74	R	41	60		
3	2	R	74	73	R	46	62	nearly unchanged	
4	5	R	73	72	R	62	61		
5	2	R	73	72	R	40	62		
6	2	R	73	72	R	42	61	0	2
7	5	R	71	70	R & A	31	43	0.4	2.2
8	2	R	71	70	R & A	28	38		
9	2	R	71	69	R & A	32	37		
10	5	R	70	68	R & A	25	28		
11	2	R	71	68	R & A	22	26		
12	2	R	70	68	R & A	15	30	0.16	1.5
13	5	R	69	68	R	3	5	0.2	2.4
14	2	R	68	65	R	2	2		
15	2	R	66	63	R	1	1		
16	5	R	64	62	R	0.5	0.5		
17	2	R	60	60	R	0	0	0.4	1.2
18	2	R	60	59	R	0	0	0.27	0.85 Total

for recovery. In fact, (period No. 13), the depression was still progressing at a rate about ten times faster than the normal.

The tissue did not recover its contractility during the time of the experiment. Thus, 2-aminobenzothiazole had a depressing action on striated muscle. This may have occurred either through a direct action on the cells or through the peripheral nerve endings.

The application of the saline solution of the agent to the conjunctiva of the rabbit produced neither myosis nor mydriasis. There was no sign of local irritation, and the light reflex was unimpaired. The results were confirmed on two other animals. The corneal reflex was retained and there was no local anesthesia. The low concentration of the solution may account for its inactivity.

Allusions have been made in the literature to the chemical resemblance between the quinoline and benzothiazole nuclei, and to the possibility of their pharmacological similarity. With this in mind, some preliminary experiments were made on the effect of 2-aminobenzothiazole on the uric acid in the blood of the rabbit and the cat.

In the rabbit, immediately after a control sample of blood was taken from the ear vein or the heart, 50 mgs. per kilogram of the aminobenzothiazole in saline solution were injected intraperitoneally. At definite intervals, thereafter, samples of blood were analyzed. In every case, there was marked rise in the uric acid content of the blood. This usually occurred within two hours after the administration of the drug. In one animal, the urine was collected also. When tested with sodium hypochlorite, it showed an excretion of the drug after the second hour and for three hours. In a special experiment, at exactly the same hours on each of two successive days, samples of blood were taken from non-fasting animals. On the second day, 50 mgs. per kilogram of animal were injected intraperitoneally immediately after taking the first sample of blood. The controls of the first day showed a normal rise of 29% and 59% in the uric acid content in two hours; while the tests of the second day showed a rise of 130% and 100% respectively. The injection of saline alone caused a fall of 10%.

In the cat, the uric acid content of the blood was also determined before and after the injection of the agent. Here, the rise in uric acid content was 35%.

Although these results were of a very preliminary nature, they warrant a further study of this phase of the action of 2-aminobenzothiazole. However, in view of the fact that cats and rabbits excrete a part of their purines in the form of allantoin, further research in this direction might better be made using birds (chickens, pigeons), or the Dalmatian hound, as recommended by Benedict.[15]

Besides controls on manipulation, and with saline, it would be essential to run comparative experiments with 2-phenylcinchoninic acid, which increases the elimination of uric acid. Since the Folin procedure for analyzing the blood requires the withdrawal of at least 4 cc. of blood for each analysis, a micro method would be preferable.

The author wishes to express her appreciation of the opportunity to use the laboratory facilities and some reagents at the New York Homeopathic Medical College and Flower Hospital.

SUMMARY OF THE PHARMACOLOGICAL EFFECTS OF 2-AMINOBENZOTHIAZOLE

1. The systemic effects consisted of collapse, convulsions, rapid and shallow breathing,—due apparently to failure of circulation and respiration. No specific symptoms were noted.

2. The effect on respiration.

a. The effect of diluted acid on the rate and depth of respiration in the cat:

Doses of hydrochloric acid solution (N/22) greater than one cubic milliliter per kilogram of animal produced a much greater depth of breathing, while the rate was unvaried in all doses tried.

b. The effect of the agent dissolved in the same concentration of acid and administered in the same volume-doses:

The rate was accelerated and the amplitude was diminished. The total ventilation was practically unaltered. Overdoses killed by respiratory paralysis.

c. In each of the six experiments with a non-acid solvent (saline), the effect on the respiration in the cat and in the rabbit was in the same general direction as in the case of the acidic solution (2b), but owing to the low concentration attained at any moment, the results were not so decisive.

3. The effect on the heart and circulation.

The blood pressure was lowered; ultimately the vasomotor center was paralyzed. The pulse rate was affected very little, excepting in overdoses. The heart muscle was practically unaffected by the agent.

4. Approximately 16 milligrams per kilogram of animal are the maximum therapeutic dose intravenously.

5. Striated muscle lost its irritability promptly and completely.

6. The uric acid of the blood was apparently increased.

GENERAL SUMMARY OF THE ACTION OF BENZOTHIAZOLE AND OF 2-AMINOBENZOTHIAZOLE

1. Compounds containing the benzothiazole nucleus produce initial stimulation of the respiratory center (after small doses) followed by depression and paralysis of the same (after large doses).

2. These compounds have very little effect on heart muscle or on the pulse, excepting in toxic doses. However, they always cause lowering of the blood pressure (after large doses) with ultimate paralysis of the vasomotor center. The heart fails through asphyxia and not through a direct action of the agent.

3. In their general action, benzothiazoles produce symptoms of collapse, or stupor (convulsions), rapid and shallow breathing, and increased reflex irritability.

4. Benzothiazole has an irritant action which may become vesicant on prolonged application. The action may be delayed, and sensitization may be increased with repeated applications. Man is markedly susceptible to this compound.

5. Special reactions are found in the production of anuria by benzothiazole in the rat; depression of contractility of striated muscle by 2-aminobenzothiazole in the frog; and increased uric acid in the blood by the same agent in the rabbit and the cat.

CONCLUSIONS

A. Chemistry.

The hydrochloride of o-aminothiophenol and the 2-anilinobenzothiazole have each been prepared by new methods.

The new benzothiazoles which have been synthesized consist of the following:

2-phenyl-5-cyanobenzothiazole,
2-phenylbenzothiazole-5-carbamide,
Nitro-2-phenyl-5-cyanobenzothiazole,
2-phenylbenzothiazole-5-carboxylic acid, and its methyl ester.

None of these compounds possess any odor, or any marked rubefacient action. All are colorless. Aside from the very low solubility of 2-nitro-4-cyanophenyldisulphide in glacial acetic acid, rendering the formation of the zinc salt of 2-amino-4-cyanothiophenol somewhat difficult, the reactions proceeded smoothly. The benzothiazoles with the cyano and carboxylic acid groups in the 5-position were readily crystallizable from alcohol, in contrast to those in the 6-position.[21] The melting points of the 5-carboxylic acid and its methyl ester were notably higher than those of the corresponding 6-isomers. Otherwise, no striking difference was noted between compounds having the substituents in the 5-position and those in the 6-position.

B. Pharmacology.

From the foregoing experiments, it is evident that the benzothiazole nucleus, containing cyclic, divalent sulphur, has a slight pharmacological action. By the use of a compound soluble in physiological saline and yielding a solution having a concentration of hydrogen ion practically the same as that of blood, precipitation or physical incompatibility have been avoided. By the use of the Murphy drip the rate of injection has been slow and uniform.

The major action of the drug is on the respiratory center. The heart fails through asphyxia and not from the direct action of the drug. The very mild action of therapeutic doses on respiration and circulation, together with the interesting observations on the changes in blood uric acid warrant a further study of 2-aminobenzothiazole in the expectation of the development of a derivative of benzothiazole which may be of value in therapeutics.

C. Chemotherapy.

A correlation between chemical constitution and antiseptic action can be made only to a very limited extent. Thus, this action is associated with the mother substance itself and not solely with the 2-amino derivative, the anils or styrils.[42]

The vesicant action is most notable in benzothiazole and in dehydrothio-p-toluidine, being practically absent from 2-aminobenzothiazole and from the new derivatives with substituents in the 2:5-positions. In view of the fact that this phenomenon is produced by many chemically dissimilar substances, no correlation is possible at present. However, the difference in action between the 2:6 compound and the 2:5 or 2-derivatives is suggestive.

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